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Assessment of watercore development in apples with MRI: Effect of fruit location in the canopy

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ABSTRACT

Watercore distribution inside apple fruit (block or radial), and its incidence (% of tissue) were related to the effect of solar radiation inside the canopy as measured by a set of low-cost irradiation sensors. 221 samples were harvested in two seasons from the top and the bottom of the canopy and submitted to the non-invasive and non-destructive technique of magnetic resonance imaging (MRI) in order to obtain 20 inner tomography slices from each fruit and analyze the damaged areas using an interactive 3D segmentation method. The number of fruit corresponding to each type of damage and the relevant percentage were calculated and it was found that apples from the top of the tree were mainly of the radial type (84%) and had more watercore (approx. 5% more) than apples from the bottom (65% radial). From the image segmentation, the Euler number, a morphometric parameter, was extracted from the segmented images and related to the type of watercore symptoms. Apples with block watercore were grouped in Euler numbers between −400 and 400 with a small evolution. For apples with radial development, the Euler number was highly negative: up to −1439. Significant differences were also found regarding sugar composition, with higher fructose and total sugar contents in apples from the upper canopy, compared to those in the lower canopy location. In the seasons studied (2011 and 2012), significantly higher sorbitol and lower sucrose and fructose contents were found in watercore-affected tissue compared to the healthy tissue of affected apples and also compared to healthy apples.

1. Introduction

Watercore is a physiological disorder affecting apples and pears (Kajiura et al., 1976; Yamaki et al., 1976, 1977; Inomata and Suzuki, 2001), involving the appearance of fluid-filled intercellular spaces with elevated sorbitol rates (Marlow and Loescher, 1985). In some varieties and in some parts of the world, such as Japan, it is considered as an indicator of full ripeness and they are called 'honeyed apples' (Kasai and Arakawa, 2010).

Although this disorder was described a long time ago (Evans, 1909; Faust and Williams, 1969), its physiological mechanism is only partially understood. Several authors have described physiological and biochemical factors affecting watercore: for example, Marlow and Loescher (1985) found that sorbitol dehydrogenase activity increased during maturation but no relationship was found between the susceptibility of watercore development and the activity of this enzyme. Wang and Faust (1992a) determined that apples affected by watercore produce more ethylene and had higher contents of polyamine compounds. Wang and Faust (1992b) stated that fruit with watercore had more glycolipids, and that the membrane lipids were altered in watercore-affected fruit. Bowen and Watkins (1997) found higher internal ethylene concentrations, soluble solid content and dry matter, sorbitol and sucrose and lower contents of glucose and fructose in apples with higher watercore symptoms. Flesh firmness, starch and calcium concentrations were also lower in slight watercore incidences but increased again in moderate to severe watercore cases. Gao et al. (2005) proposed that sorbitol accumulation in the intercellular space is related to a decrease in the ability to transport sorbitol into parenchyma tissues in late watercore apples. Nevertheless, Yamada and Kobayashi (1999) found no links between watercore and sorbitol or maturity indices (such as ethylene), membrane permeability and flesh firmness in affected and non-affected apples induced by preharvest fruit temperature. Kweon et al. (2012) linked watercore disorder to fruit maturity.

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Other authors have studied the possible causes of watercore development: Yamada et al. (1994) observed that temperature differences between day and night are not required for watercore development. Yamada et al. (2004) established that apples exposed to low temperatures developed watercore. At 25 °C almost no watercore was observed and water potential gradually decreased. Another study carried out by Yamada et al. (2006) suggests that fruit from the outer part of the canopy had higher watercore incidence in the immature stages (mid-August) and that the sorbitol content variation was parallel to this period. Also, they found higher amounts of sorbitol, fructose and glucose in the vacuoles of cells in the outer canopy. They propose that sorbitol metabolism and uptake might occur in watercored apples during the immature stage. Also, Yamada et al. (2010) confirmed that active phloem transport might be induced by the increased partitioning of assimilates and not by the higher potential of leaf photosynthesis.

Two distinct forms of watercore have been identified, block and radial watercore (Clark et al., 1998), which seem to be related with the growing region (Harker and Watkins, 1999). In some varieties, if the fruit is very affected, external symptoms may be noticed. But block watercore and some radial watercore affected fruit do not present external symptoms (Ferguson et al., 1999). This is where the importance of a non-destructive method for watercore detection lies.

Magnetic resonance imaging (MRI) is a technique that permits watercore detection without destroying the sample. Numerous authors have used MRI for the evaluation of internal quality and fruit properties (Hernández-Sánchez et al., 2004, 2006; Zhang and McCarthy, 2012) and, in particular, several studies have used MRI for the detection of this disorder (Wang et al., 1988; Clark et al., 1998; Clark and Richardson, 1999; Cho et al., 2008; Marigheto and Hills, 2005).

Preliminary experiments showed the feasibility of using NMR and MRI for driving sample selection for HR MAS NMR analysis (Melado-Herreros et al., 2013). In this work, significant changes in specific sugars were found for affected tissue compared to sound, even in areas with no visible changes, addressed by means of MRI.

To our knowledge, no one has previously related watercore incidence to solar radiation, although Yamada et al. (2006) observed higher watercore incidence in apples from the outer canopy than those from the inner canopy, which might suggest that solar radiation may interfere in any metabolic route that causes this disorder.

In the present work, two different kinds of low-cost solar radiation sensors (based on the photovoltaic effect) were used on apple trees ('Esperiega' variety, in which watercore development is a desirable characteristic as it increases marketability). The aim was to measure the penetration of solar radiation in the upper and lower sections of the tree canopy, in two consecutive years. The experiment was carried out over the course of 1 month before the harvest date.

The objectives of this work were (i) the use of a non-destructive technique, such as MRI tomography as a means for addressing 3D development of watercore symptoms; (ii) to study the relationship between direct solar radiation incidence and the development of watercore symptoms.

2. Materials and methods

2.1. Plant material

Fruit from 18-year-old 'Esperiega' apple trees (*Malus domestica*) were harvested in two seasons at a commercial orchard located in Ademuz (Valencia). In the first season, 146 apples were picked randomly within the orchard (67 from the bottom of the canopy, located approximately at a height of 1.5 m, and 79 from the upper part of the canopy, located at a height of approximately 3 m). In the second season, a total of 75 apples were picked randomly from the orchard (46 from the lower canopy and 29 from the upper canopy).

2.2. Solar radiation penetration in the canopy

Two different kinds of sensors were used to assess penetration of solar radiation within the canopy, one in each season. In both cases, the maximum current obtained from solar cells (short circuit current) was used to determine the solar penetration. These currents were measured and recorded in a data acquisition system based on two USB data acquisition boards controlled by a computer in the first season, and a data-logger in the second one. In both cases, the measurements were obtained on the basis of mean values every 1 and 2 min, respectively.

During the first season (September 2011), 14 low-cost solar mini-modules manufactured by Sanyo were used. The model selected was AM-5608, consisting of 6 amorphous silicon (a-Si) based cells, connected in series. A sensor was positioned at the bottom (1.5 m height) and at the top (3 m height) of six trees; two were positioned as a reference over the tree canopy on a dedicated support, exposed to sunlight. All the sensors were mounted horizontally, on the branches of the trees, inside the canopy. This equipment was placed in the field for 26 days. The disadvantage of these sensors is that they present a partial shadow effect, as the cells are connected in series and if one of them are shadowed it is not possible to distinguish how many of the cells were shadowed due to the effect does not depend on how many cells are shadowed. For this reason, the data obtained from this set up were considered qualitative data.

In the second season (September 2012) single solar cells manufactured by IXOLAR were used. In this case the technology of the solar cells was crystalline silicon (c-Si) and the model KXOB22-12X1. The location of the sensors was the same as described above. This experiment was conducted over a 27-day period.

Both sensor technologies were useful for the experiments, but the second option is considered a better solution not only for a qualitative analysis but also for a quantitative one. A detailed and extended description of sensor calibration and testing will be published in the near future.

2.3. Sugar analysis methodology

Ten fruit from the 2011 harvest and another ten from 2012 were selected in order to study the sugar content of both healthy and affected apples from the top and the bottom of the canopy.

Fruit were peeled with a sharp knife; in total, 12 tissue samples in 2011 and 16 in 2012 were achieved, corresponding to sound fruit and sound tissue and affected tissue from watercore fruit, respectively. In the apples from the 2012 harvest, the position on the tree was also taken into account in the sugar analysis.

Flesh was weighed, immediately frozen separately in liquid nitrogen, and stored at −20 °C until analysis. The frozen fruit material (5 g) was homogenized in a Polytron with 10 mL of extraction solution consisting of ethanol/Milli-Q water (80%, v/v). The mixture was centrifuged at 20,000 × *g* for 20 min at 4 °C. The supernatant was recovered and processed to be assayed by high-performance liquid chromatography (HPLC) as described by Cantín et al. (2009), with some modifications. To estimate the variation in the sugar profile among different fruit either affected or otherwise by watercore, sugar composition and quantification were measured as described by Cantín et al. (2009). For the analysis, 250 μL of the homogenized extract was incubated at 80 °C for 20 min in 200 μL of 800 mL/L ethanol, with 5 g/L manitol added as an internal standard. Samples were purified using ion exchange resins (Bio-Rad Barcelona, Spain) (Jimenez et al., 2011). Twenty (20) μL was injected into the

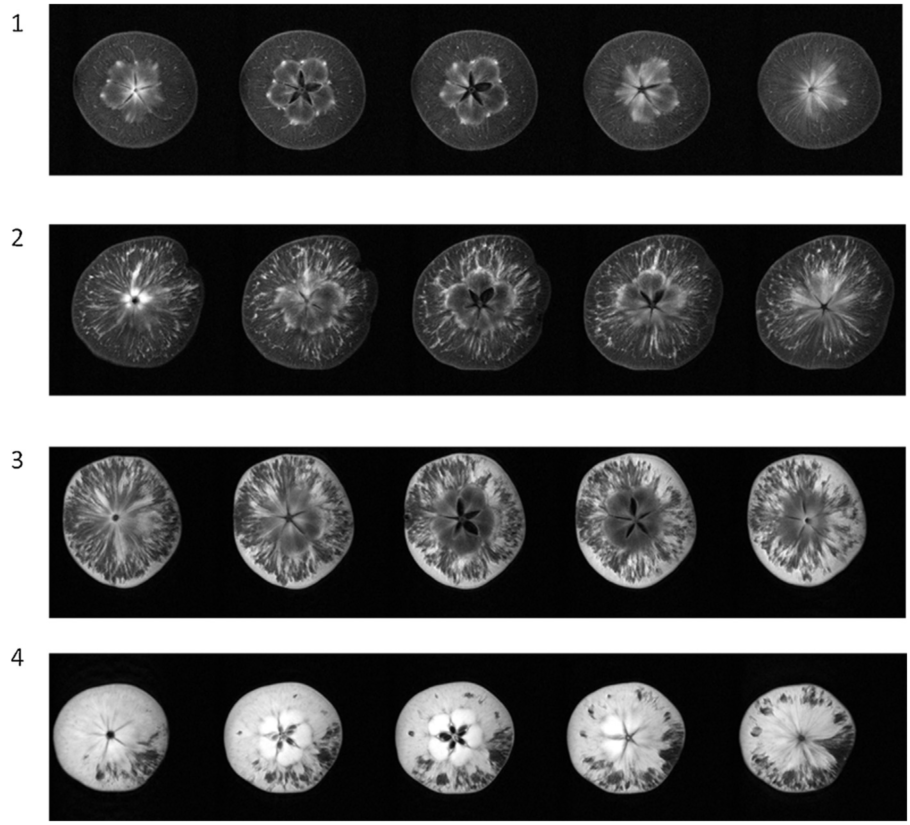

Fig. 1. Central slices of MR images belonging to the four watercore levels, classified by three experts. (1) Sound apple; (2) light watercore; (3) medium watercore; (4) strong watercore.

HPLC system (Aminex HPX-87C column, 300 mm × 7.8 mm; Bio-Rad, Barcelona, Spain) with a refractive index detector (Waters 2410). The solvent was deionized water running at a flow rate of 0.6 mL per min at 85 °C. Sugar quantification was performed with Millenium 3.2 software (Waters) using analytical grade standards (Panreac Quimica SA, Barcelona, Spain). Sugar concentrations were expressed as mg per g FW (fresh weight).

2.4. MRI experiments

Magnetic resonance experiments were carried out at the Nuclear Magnetic Resonance Research Assistance Centre (CEI-Moncloa) dependencies on a Bruker BIOSPEC 47/40 (Ettlingen, Germany) spectrometer, operating at 200 MHz. All experiments were performed under static conditions, with an actively shielded imaging gradient set and RF volume coil with an inner diameter of 20 cm. The bore of the magnet is horizontal, 147 cm long and with 40 cm diameter.

MRI screening was carried out in 2D T2-weighed Rapid Acquisition with Relaxation Enhancement (RARE) sequences, with a view to obtaining all the images for visual inspection. Coronal images (x–z plane) were obtained from apples placed with their central axis along the y-axis of the magnet. The MRI sequence parameters were: recovery time (TR) 5000 ms, echo time (TE) 60 ms. The field of view (FOV) and the slice thickness used were 8 cm × 8 cm and 3 mm, respectively. All images were acquired in 32 bits, with 256 × 128 acquisition matrix size and reconstructed to 16 bits with zero filling to 256 × 256 and with the same scale. The total acquisition time was 2 min 2 s and 20 slices were obtained for each apple.

From the MR images, the eight central slices of each apple were chosen by three specialists to classify the apples (Fig. 1) into four classes, depending on their apparently watercore level (1 – sound apples; 2 – light watercore; 3 – medium watercore; 4 – strong watercore) and indicate the type of watercore development (C – block; R – radial) (Fig. 2). The total numbers of apples and watercore stages, together with position on the tree, are provided in Table 1.

2.5. Image processing

Prior to morphometrical analysis, the area affected by watercore was segmented. Traditionally, literature on MR image segmentation has been divided into two categories: grey scale segmentation

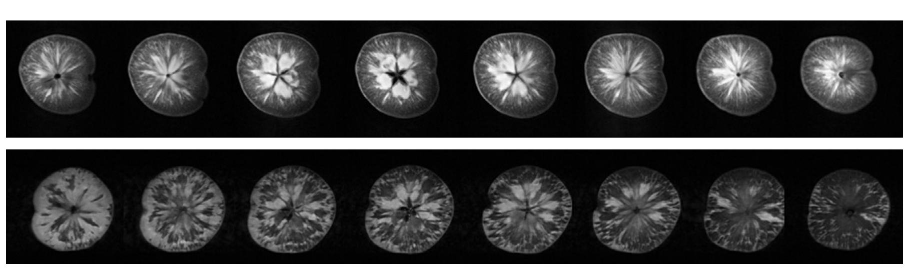

Fig. 2. Examples of a tomography of two different apples that have block (on the top) and radial (on the bottom) watercore.

Table 1
Number of apples classified by their watercore level (sound, light, medium, strong), type of watercore development (C – block watercore; R – radial watercore) and position on the tree (top and bottom).

Position	Sound	Light		Medium		Strong		Total
	–	C	R	C	R	C	R	
Top	23	5	26	1	34	8	11	108
Bottom	22	12	31	7	28	12	1	113
Total	45	17	57	8	62	20	12	221

(which involves several types of methods, such as edge detection, boundary tracing, thresholding, seed-growing, template models or random field) and multi-spectral image segmentation (which includes pattern recognition and algebraic approaches as supervised methods, with clustering as the unsupervised method) (Clarke et al., 1995). As watercore is a disorder characterized by free water in the tissues, on T2 weighed images the affected area will appear brighter than the rest of the tissue and thus, apparently, the most straightforward technique to segment would seem to be thresholding-based segmentation methods. Nevertheless, this method poses several problems (Clarke et al., 1995), as it works properly for severely affected apples, but not so much for slight watercore stages, especially for apples with radial watercore, as it thresholds pixels belonging to non-affected tissue but with slight water accumulation. Thus, in this work, where the analysis of watercore symptoms within the fruit is a major issue, automated segmentation was disregarded in favour of a 3D interactive segmentation method applied to each of the 20 slices of every apple, using the software Avizo Fire Edition 7.0 (VSG, Bordeaux, France).

The morphometric parameters considered were the area of affected tissue (μm^2), the total area of apple tissue (μm^2) and the Euler number, which is an indicator of the connectivity of a 3D structure and topology. The Euler number for a 3D structure is a scalar defined as the total number of objects segmented in the image minus the number of redundant connections or loops, also referred to as connectivity or genus (Vogel and Roth, 2001) in those objects plus the number of enclosed cavities (Gundersen et al., 1993; Kroustrup and Gundersen, 2001). The first term of the Euler number defines a 1D structure, while the second one defines a 2D structure. For a 3D structure, the first term refers to the number of particles and the other two take into account the complexity of the particles (Gundersen et al., 1993). Large negative or positive Euler number values indicate poorly connected structures, while medium negative and positive values indicate compact but connected structures. Low positive Euler number values indicate several better connected segmented objects. The percentage of affected tissue for each apple is computed as the area of affected tissue over the total apple tissue.

2.6. Statistical analysis

In order to validate the solar radiation acquisition system, an analysis of variance (ANOVA) was performed on the data collected in 2012, as the data from 2011 give a qualitative idea of the solar radiation but are not suitable for quantitative analysis (further discussion on this matter is provided later in the text).

In order to understand watercore development according to solar radiation incidence, another ANOVA was performed on both the Euler number and the fruit damaged area (%), depending on the type of damage (block or radial watercore), and the class (2, 3 or 4). Class 1 was not considered as it cannot develop any type of damage and because of the position of the samples on the tree.

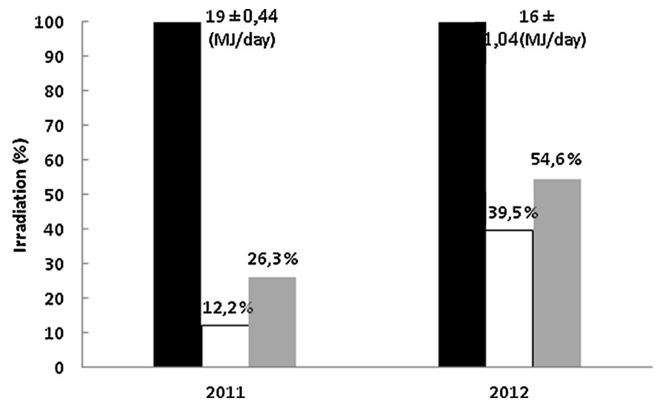

Fig. 3. Comparison of the percentage of global irradiation (■), sensors on the bottom (□) and sensors on the top (▩) of the tree for the two kinds of solar cells on both seasons (2011 and 2012). Cells from 2011 have lower irradiation percentage as the configuration of these cells is used in series and it shows a problem when partial shadowing.

3. Results and discussion

3.1. Solar radiation penetration in the canopy

Fig. 3 shows an evaluation of solar radiation in the reference position, above the canopy, in 2011 compared to 2012. In the latter, a 3.3 MJ/day reduction was found. Fig. 3 also compares solar radiation in the upper part of the canopy and the lower portion of the tree. In 2011, the amount of solar radiation within the canopy at either position was much lower than that registered in 2012 (new design). The amount of solar radiation in the upper canopy in 2011 was too far from the reference radiation (26%). This fact is due to the configuration of the cells used in series, which does not allow for differentiation between the shadowing of only one cell in the mini-module and the shadowing of two or more cells. Therefore, solar penetration inside the canopy was underestimated in 2011 in both the top and bottom positions, while the sensors used in the 2012 season did not have this problem. The irradiation captured inside the canopy was higher (54.6% in the top and 39.5% in the bottom) and the data are more reliable.

The results of the ANOVA on solar radiation penetration inside the canopy (2012) show a significant effect on the top or bottom position (F = 60.9, significant at level 0.01) and on the tree (F = 31.9, significant at level 0.01). A significant effect of the interaction between tree and position was also found, with an F value of 28.9, significant at level 0.01. Fig. 4 provides the daily average solar

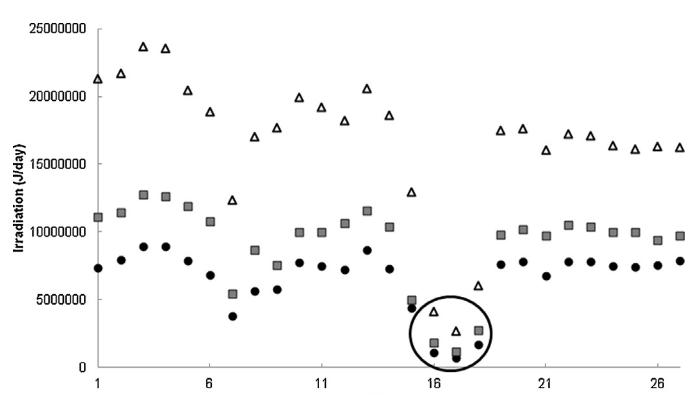

Fig. 4. Average daily irradiation expressed on J per day in function of the day of the experiment for the top (▩) and the bottom (●) of the canopy and the average global radiation (△), acquired by the reference cells on the second season (2012). Circled individuals mean data acquired on cloudy days.

Table 2
Mean sugar concentrations, on 12 tissue samples in apples from 2011 season. Comparisons at 0.05 and 0.01 level are indicated by * and **, respectively. No significant differences are expressed by ns. By rows, consecutive values of the same letter (a, b) are no significant at 0.05 level.

Sugar content (mg sugar/g FW)	Healthy apple	Watercored apple		*F*
	Healthy tissue	Healthy tissue	Watercored tissue	
Fructose	72.0^{a}	69.2^{a}	63.1^{b}	4.2*
Sucrose	28.0^{a}	31.8^{a}	15.0^{b}	19.1**
Glucose	15.1^{b}	19.5^{ab}	21.7^{a}	5.1*
Sorbitol	8.0^{b}	14.9^{b}	29.3^{a}	21.4**
Total sugar content	123.2	135.2	129.3	ns

FW, fresh weight.

Table 3
ANOVA on sugar concentrations for each sample. Comparisons significant at the 0.05 level are indicated by *. No significant differences are expressed by ns.

Sugar content (mg sugar/g FW)	*F*			Sum of squares total	Sum of squares error	Degrees of freedom
	Class	Position	Class × position			
Fructose	14.4*	11.0*	ns	818.7	148.4	15
Sucrose	9.2*	ns	ns	1509.5	421.2	15
Glucose	ns	ns	ns	202.7	143.1	15
Sorbitol	5.1*	ns	ns	1346.3	458.5	15
Total sugar content	ns	6.7*	ns	3272.0	1535.8	15

radiation during the 27-day period before harvesting. There were only 3 cloudy days (11% of days).

3.2. Sugar analysis

The ANOVA results of the mean sugar concentrations from each sample for 2011 and 2012 are shown in Tables 2 and 3, respectively, and the mean values in Table 4.

Fructose and sucrose had lower levels in affected tissues compared to sound apples in both the years 2011 and 2012, with a decrease of over 10 mg/g FW. In 2011, a fructose content of 63.13 ± 1.03 mg/g FW and a sucrose content of 15.04 ± 3.88 mg/g FW were found for affected tissues, while in 2012, 56.90 ± 1.67 mg/g FW and 18.03 ± 2.81 mg/g FW were found, respectively. Bowen and Watkins (1997) found lower levels of fructose in affected tissues compared to sound, but higher sucrose content. In contrast to these results, Yamada et al. (2006) found higher fructose and lower sucrose content in affected tissues compared to sound. General agreement was only found in terms of the decrease in sucrose content for watercore affected tissue. Yamada et al. (2006) also indicated significant higher fructose content in apples from the outer part of the canopy compared to the inside, while no significant differences in sucrose content were found between the canopy positions. In our study, a significantly higher fructose content was found in apples from the upper part of the canopy (68.2 ± 1.5 mg/g FW) compared to apples from the lower part of the canopy (61.0 ± 1.4 mg/g FW).

As regards the total amount of sugar, there were no significant differences between tissues (affected and healthy) in 2011 or in 2012, although in 2012 it was significantly higher in the case of apples from the upper part of the canopy (143.6 ± 5.1 mg/g FW) than for the lower part (125.6 ± 4.8 mg/g FW). This latter effect is consistent with results obtained by Yamada et al. (2006).

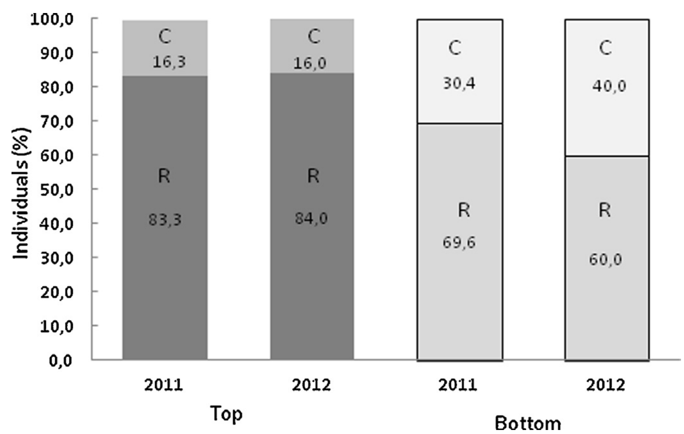

Fig. 5. Number of individuals (expressed in %) from the bottom and the top of the canopy of the tree in both seasons. Each colour is related with the type of observed damage (R – radial; C – block).

Based on the results obtained in 2011, the affected tissue showed significantly higher glucose levels (21.8 ± 1.6 mg/g FW) compared to healthy apples (15.1 mg/g FW) and to healthy tissue in affected apples (19.5 mg/g FW). Glucose levels in affected tissue were also higher in 2012 (17.5 ± 1.6 mg/g FW), but these were not significant compared to the glucose content in healthy apples (17.2 ± 2.2 mg/g FW) and healthy tissue in affected apples (17.2 ± 1.6 mg/g FW). These results are consistent with those found

Table 4
Mean values of sugar content for 2012 season, according to the type of tissue and the position on the tree of the fruits. By columns, consecutive values of the same letter (a, b) are no significant at 0.05 level.

	Fructose (mg/g FW)	Sucrose (mg/g FW)	Glucose (mg/g FW)	Sorbitol (mg/g FW)	Total sugar content (mg/g FW)
Type of tissue					
Healthy	69.0 ± 2.2^{a}	38.1 ± 3.7^{a}	17.2 ± 2.2^{a}	23.2 ± 3.9^{a}	147.6 ± 7.1^{a}
Healthy in affected	67.9 ± 1.6^{a}	28.2 ± 2.8^{a}	17.2 ± 1.6^{a}	18.4 ± 2.9^{a}	131.8 ± 5.4^{a}
Affected	56.9 ± 1.6^{b}	18.3 ± 2.8^{b}	17.5 ± 1.6^{a}	31.7 ± 2.9^{b}	124.4 ± 5.4^{a}
Position					
Top	68.2 ± 1.5^{a}	27.3 ± 2.6^{a}	19.2 ± 1.5^{a}	28.4 ± 2.7^{a}	143.6 ± 5.1^{a}
Bottom	61.0 ± 1.4^{b}	28.6 ± 2.5^{a}	15.4 ± 1.4^{a}	20.5 ± 2.6^{a}	125.6 ± 4.8^{b}

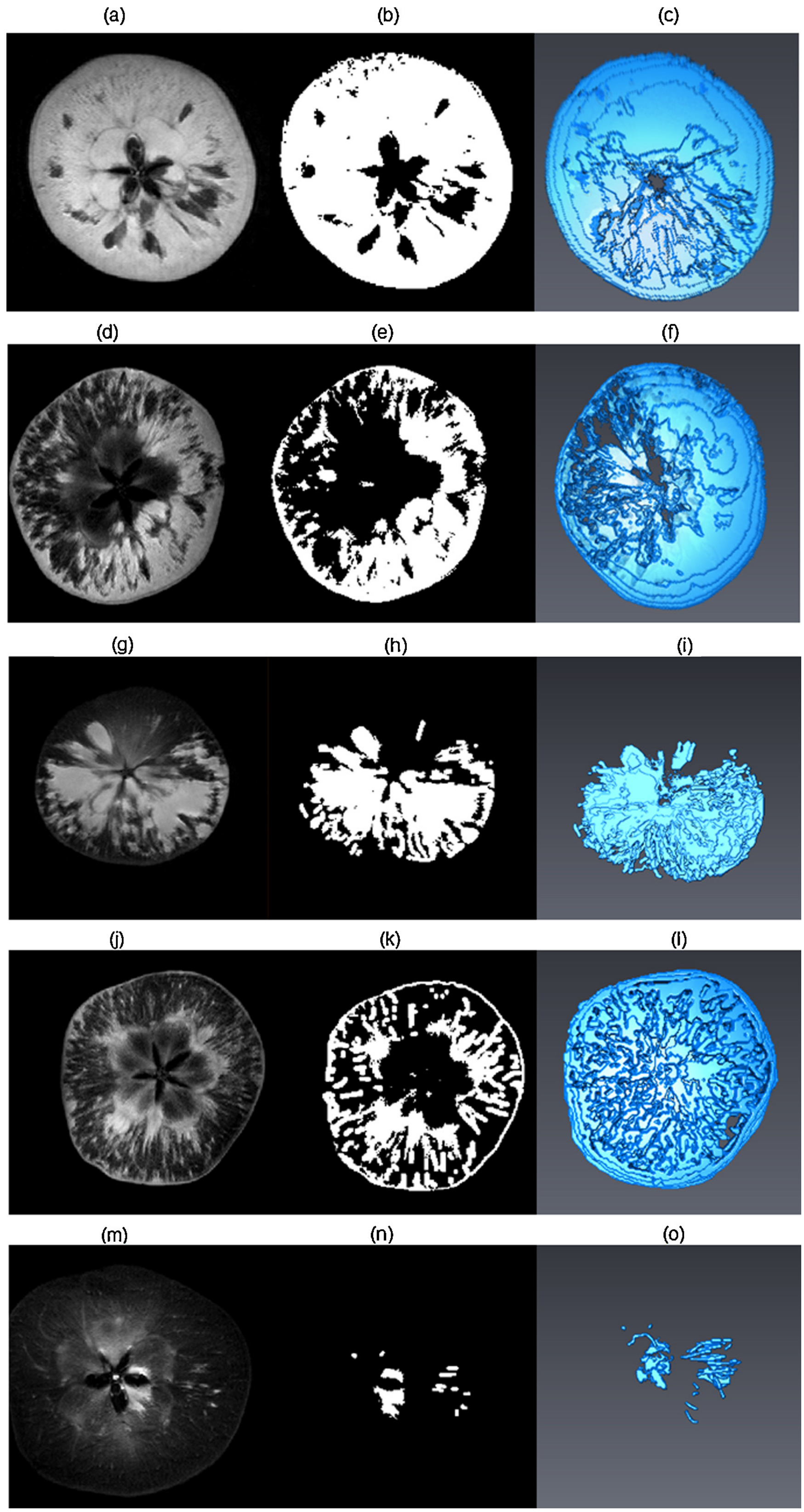

Fig. 6. MR images, (a), (d), (g), (j) and (m), segmented images (b), (e), (h), (k) and (n) and 3D reconstruction (20 slices) of the segmented area of the tomography (c), (f), (i), (l) and (o) of four images with different Euler number. Euler number (a–c) = 137 (watercore damage = 67.51%); Euler number (d–f) = −583 (watercore damage = 49.50%); Euler number (g–i) = −137 (watercore damage = 45.43%); Euler number (j–l) = −1275 (watercore damage = 35%); Euler number (m–o) = 30 (watercore damage = 0.43%).

by Yamada et al. (2006), who detected higher glucose levels in every cellular compartment, especially in the vacuoles. They also found that apples from the outer canopy, which were more susceptible to watercore development than apples from the inner canopy, had a higher glucose content. Our study reveals that apples from the top of the canopy also had a higher glucose content (19.2 ± 1.5 mg/g FW) than apples from the bottom of the canopy (15.4 ± 1.4 mg/g FW), although these latter differences are not significant.

Most of the authors who have studied watercore, such as Bowen and Watkins (1997), Yamada and Kobayashi (1999), Yamada et al. (2006), among others, have found higher sorbitol levels in affected tissues than in non-affected. In our study, sorbitol contents were higher in both seasons in affected tissues with 29.3 ± 2.3 mg/g FW in 2011 and 31.7 ± 2.9 mg/g FW in 2012, compared to healthy apples, which had 8.0 mg/g FW in 2011 and 23.2 ± 3.9 mg/g FW in 2012, and also compared to healthy tissue in affected apples, containing 14.9 mg/g FW in 2011 and 18.4 ± 2.9 mg/g FW in 2012. It is interesting to point out that our sorbitol levels far exceeded any values reported in the cited literature (approx. 7 mg/g FW in Bowen and Watkins (1997), approx. 6 mg/g FW in Yamada and Kobayashi (1999) and approx. 12 mg/g FW in Yamada et al. (2006)). It should be noted that in our analysis, watercore is considered to be an added value and not an indicator of lack of quality. Moreover, in our study, the total sugar content (around 130 mg/g FW) was much higher than reported by Bowen (approx. 75 mg/g FW) but similar to Yamada et al. (2006), approx. 150 mg/g FW.

3.3. Visual classification

In both seasons there were more individual apples with radial (R) rather than block (C) watercore. Nevertheless, a higher proportion of radial watercore was found in samples from the top of the tree: 83.3% and 84.0% of apples with radial damage from the top of the tree in 2011 and 2012, respectively, and 69.6% and 60.0% in the lower part of the canopy for both seasons, respectively (Fig. 5). Also, a slightly higher proportion of severely affected apples was found at the top of the canopy (17%) compared to the bottom (12%).

3.4. Image processing

Fig. 6 provides examples of the result of the interactive segmentation procedure, together with a 3D reconstruction of the affected areas. The corresponding values of affected tissue (67.51%, 49.50%, 45.43%, 35% and 0.43%) and the Euler number (137, −583, −137, −1275 and 30) are also included. Positive Euler numbers are found when segmented objects are unconnected which, in this case, corresponds to a slightly affected fruit. Large negative Euler numbers are found for complex segmented shapes with highly connected areas.

The ANOVA performed on the percentage of damaged tissue according to segmentation indicates a very significant effect at level 0.05 regarding visual categorization (Class), with an F value of 336.75 and also significant differences at level 0.05, according to the position on the tree, with an F value of 8.25, though the interaction between these two parameters is not significant.

As may be seen in Table 5, the apples harvested from the top of the trees presented on average a higher proportion of damaged tissue (Fig. 7) for the three categories (17.40 ± 1.90% for class 2, 47.14 ± 1.90% for class 3 and 74.53 ± 2.53% for class 4) than in apples from the bottom of the tree (10.5 ± 1.70% for class 2, 41.00 ± 1.87% for class 3 and 71.91 ± 3.10% for class 4). These apples received higher irradiation than those from the bottom and this is consistent with the findings of Yamada et al. (2006), who established that apples from the outer part of the canopy had a higher incidence of watercore. We can therefore confirm our hypothesis that watercore incidence is related to solar radiation, now that it has been quantitatively evaluated. However, the difference in affected tissue for fruit in the upper and lower positions inside the canopy decreased as the watercore stage increased (6.87%, 6.13% and 2.62%). This fact is also consistent with the findings of Yamada et al. (2006), who stated that differences were found at immature stages (mid-August).

Table 5
Mean values of damaged tissue. Values marked by letter ^{a} are significant for visual categorization (Class) at level 0.05. Values marked by letter ^{b} are significant according to the position on the tree at level 0.05.

Class	Position on the tree	Average amount of damage (%)
2	Top	17.40 ± 1.90$^{a\ b}$
3	Top	47.14 ± 1.90$^{a\ b}$
4	Top	74.53 ± 2.53$^{a\ b}$
2	Bottom	10.5 ± 1.70$^{a\ b}$
3	Bottom	41.00 ± 1.87$^{a\ b}$
4	Bottom	71.91 ± 3.10$^{a\ b}$

As already explained, the Euler number refers to the amount of segmented objects minus the number of holes plus the enclosed cavities. Significant differences are found for the Euler number according to the type of damage (F = 14.44) with average values of: −267.80 ± 36.87, −460.10 ± 37.41 and 11.61 ± 56.33 for Classes 2, 3 and 4, respectively. In incipient watercore development, several very small and unconnected examples of damage were found, with no enclosed cavities. Therefore, this means that the Euler number is a low positive or slightly negative one. In the intermediate stages, the number of holes and enclosed cavities within the damaged tissue is very relevant (very negative Euler number). The larger the damage becomes, the larger the number of segmented objects with little to no holes and thus, the Euler number once again evolves accordingly towards positive Euler numbers. No significant differences were found for the Euler number according to the position on the tree, nor for the interaction among factors. However, the type of watercore development is linked to the Euler number. Thus, when representing the percentage of damaged tissue in terms of the Euler number, apples with block watercore are grouped in Euler numbers between −446 and 400. Apples with intermediate damage (20–60%) are of the radial type and exhibit very negative Euler numbers (in many cases below −800), referring to highly complex and interconnected structures, typical of the kind of damage involved. There are two outliers that are classified as block while having a very low Euler number (−1005) together with a percentage of damage of around 45% which might be due to an error in visual classification by the experts. As a whole, apples affected by radial watercore appear on a wider range of both Euler number (from 400 to −1439) and percentage damage (from 0.3% to 89.2%).

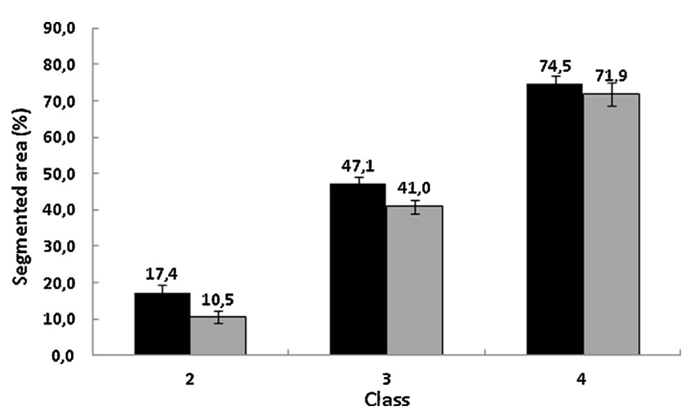

Fig. 7. Mean segmented damage on both positions, the top of the tree (■) and the bottom (□) of the tree. Apples harvested from the top of the tree have higher segmented damage than apples from the bottom.

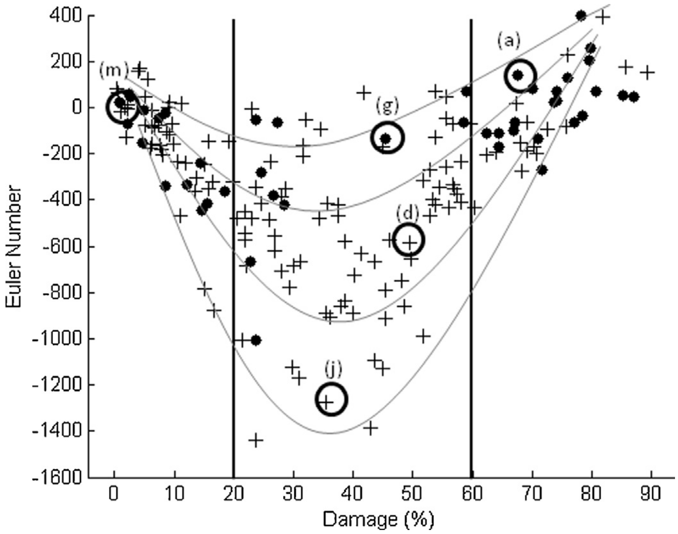

Fig. 8. Euler number against damage (%) on samples of apples. (●) represent apples with a block watercore development and (+) represent apples with a radial watercore development, that appear spread on a wider range than block watercore. Samples surrounded by a circle correspond to those showed previously in Fig. 5. Trend lines point different 3D evolution of disorder. Letters (a), (d), (g), (j) and (m) identify MRI images in Fig. 5.

This means that radial watercore has more variability in terms of the connectivity of the damage, contrary to block watercore, which evolves according to a different trend (see marked lines in Fig. 8); the letters in Fig. 8 refer to apples presented in Fig. 6.

4. Conclusions

Solar radiation within the tree canopy was measured by means of a dedicated solar cell-based sensor design. The serial configuration of the cells used in the first season meant it was not possible to obtain quantitative data, as it was impossible to distinguish between partial shadowing and total shadowing. Hence, in the second season a different set of cells was used and the data obtained were used as quantitative data: it was proven that the cells located at the top of the canopy received more irradiation (54.6%) than those located on the bottom (39.5% of daily radiation).

From the sugar analysis, significant higher sorbitol levels and lower sucrose and fructose contents were found in affected tissues compared to sound tissues from both healthy and affected apples. Sugar analysis provided higher glucose content in affected tissue in the first season, but no significant differences were found in the second season, although affected tissues presented a slightly higher glucose content. The total sugar amount did not reveal significant differences in any season in terms of watercore incidence. From the analysis performed in the second season, higher total sugar and fructose contents were found in apples from the upper rather than the lower part of the canopy.

Interactive segmentation was successful in order to achieve the 3D reconstruction of the damage. Apples from the top of the canopy presented a higher probability (>80% approximately) of being affected by radial damage and also a higher percentage of damage (17.40 ± 1.90% for class 2, 47.14 ± 1.90% for class 3 and 74.53 ± 2.53% for class 4) than apples from the bottom of the tree (10.5 ± 1.70% for class 2, 41.00 ± 1.87% for class 3 and 71.91 ± 3.10% for class 4). A higher amount of affected apple tissue (%) was found for the upper canopy compared to the lower part of the tree, although differences decrease for increasing watercore stages 2, 3 and 4, respectively: 6.87%, 6.13% and 2.62% (difference in percentage of affected fruit for classes 2, 3 and 4 between the upper and the lower canopy).

Intermediate watercore levels mainly correspond to radial watercore. The type of watercore (radial or block) can be characterized by means of the Euler number, as radial watercore has, in general, more negative Euler values than block watercore, as is to be expected for a more complex distribution of affected tissue with high connectivity. A 2D representation of damage versus Euler number provides relevant information on the stages of watercore development in the fruit.

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